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54 Adduct of mutually stabilizing menadione and thiamine.

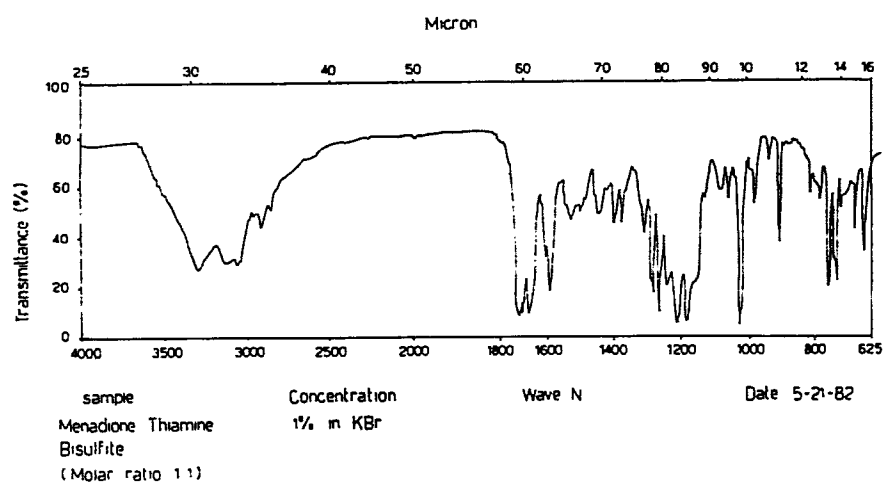
57 An adduct of menadione and thiamine is described having the general formula $C_{34}H_{36}N_4O_{11}S_3$, molecular weight 772,84 and containing the components in a molecular ratio of 2:1 respectively.

This adduct is particularly intended to be added to integrator mixtures for zootechny.

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FIG.5



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ADDUCT OF MUTUALLY STABILIZING MENADIONE AND THIAMINE

The inventor of the present invention had already described, in a preceding application (Italy No. 206008 A/78), new adducts of compounds having K-vitaminic (menadione sodium bisulfite) activity, which were stabilized by compounds also endowed with therapeutical, 5. particularly vitaminic, activity.

Said adducts represented a remarkable improvement in comparison with other previously used K-vitaminic adducts, wherein the stabilizing portion of the adduct was inert from the therapeutical point of view.

10. One of the stabilized adducts, particularly claimed in the above

mentioned Italian application, was the stabilized menadione sodium bisulfite and thiamine hydrochloride adduct, said components being present in a molecular ratio of 1:1 respectively.

- This menadione sodium bisulfite-thiamine adduct has the general
5. formula $C_{33}H_{27}ClN_4O_6S_2$, a molecular weight of 555,17 and therefore a theoretical content of menadione ($C_{11}H_8O_2$) and thiamine, this latter expressed as thiamine hydrochloride (i.e. $C_{12}H_{18}ClN_4O_2S$), of 31,0% b.w. and 60,8 b.w. respectively. This means that the above adduct derives from a mole to mole reaction
 10. between menadione sodium bisulfite and thiamine hydrochloride, with elimination of one molecule of sodium hydrochloride.

As aforesaid, the stabilization of said adduct, particularly by means of another compound, also having a therapeutical activity, was claimed in the above mentioned application.

15. Further intimate experimental studies about said adducts surprisingly led to identify another compound, which was also obtained by reacting menadione sodium bisulfite with thiamine hydrochloride, but in a ratio of 2:1 respectively; namely the elimination of two sodium hydrochloride molecules and the addition of menadione
20. bisulfite with thiamine rest takes place; so that this new adduct has the formula $C_{34}H_{36}N_4O_{11}S_3$, molecular weight 772,84 and menadione and thiamine hydrochloride equivalent contents of 44,56% b.w. and 43,64% b.w. respectively; therefore it clearly differs from the above adduct in the composition. It differs also from the
25. above adduct in another decisive characteristic: namely a mutual stabilization sets up between the adduct portions, so that the

K-vitamin stabilizes the thiamine and at the same time the thiamine stabilizes the K-vitamin.

Thanks to said characteristics, the product according to this invention represents a clear progress in comparison with the
5. product of the preceding application, particularly as regards its use in the field of feed preparation, namely of the "integrators to the animal feeding".

Indeed both active principles, expressed as menadione and thiamine hydrochloride, the presence of which is essential in feed
10. integrators, are generally added to these latter in the same amounts, by weight, and separately, so being dispersed into a medium, which is particularly apt to aid the loss of their respective activities.

On the contrary the use of the new product, subject of the present
15. application, represents an important improvement (in this particular field) in that it allows on the one hand the contemporaneous introduction - namely in one only and precisely dosed addition - of both components, in the integrator; on the other hand the introduction of an adduct in the integrator, the components of
20. which are preliminary stabilized. In this way the integrator preparation is simplified and improved and, at the same time, the loss in course of time of the relative activities is reduced.

Actually an intramolecular stabilization (IMS) is carried out, namely, a chemical IMS, which, as supposable, makes the biological
25. activity speedy and more active; as an alternative to the extra

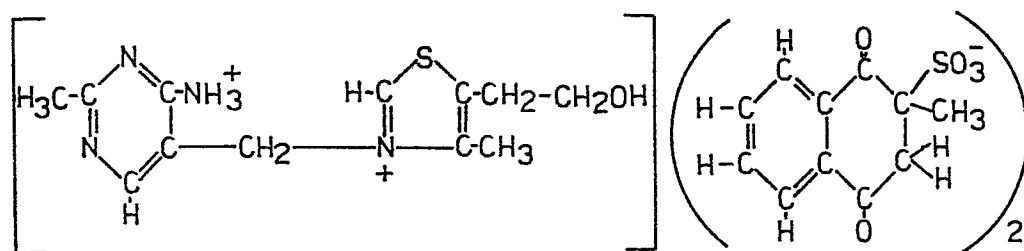
molecular stabilization (EMS), which is obtained by a physical covering operation of the product granules by a suitable protective membrane.

Consequently not only a technical, but also an economical advantage

5. in the fed preparation is attained in this way.

To recapitulate, the adduct of this invention, having the general formula $C_{34}H_{36}N_4O_{11}S_3$, theoretically contains 44,56% of menadione and 43,64% of thiamine (expressed as thiamine hydrochloride) and is better described by its structure formula, from which it results

10. that this adduct is a 3-(4 amino-2-methyl-5-pyrimidinyl-methyl) 5-(2-hydroxyethyl)-4-methylthiazole-bis (1-4-naphthoquinone-2-methyl-bisulfite).



For convenience, however, the adduct according to the present invention will be hereinafter called BMV.

15. The adduct BMV is prepared according to the process which is illustrated in the following examples, which should not be considered as limiting this invention.

Example 1 - Laboratory preparation of the product in a pure form.

A solution, consisting of 100g of menadione sodium bisulfite
20. (menadione-title 51,5%) and 200g of water, is added with a solution consisting of 50g of commercial thiamine hydrochloride (title 97%) and 50g water, within about 10 minutes of agitation, at a temperature not higher than 30°C. After about 10 minutes since the

- addition of the thiamine solution was completed, the reaction product starts precipitating and, after 30-40 minutes of curing, under stirring, the product is filtered and washed until all hydrochlorides are off the filtrate; a white solid is so obtained,
5. which is then vacuum dried at 40°C up to a constant weight and then analyzed.

The chemical analysis gives:

- menadione (2 methyl 1-4 naphtho-quinone) 44,54%
- thiamine (expressed as hydrochloride) 43,63%
10. sodium hydrochloride trace
- water absent

- The so obtained product melts (with decomposition) at 188°-190°C. It shows a characteristic I.R. spectrum, as from figure 1 and from the comparison of the same with the spectra of figures 2-5, which
15. represent:

Fig. 2 the I.R. spectrum of simple physical mixture of menadione sodium bisulphite and thiamine hydrochloride.

Fig. 3 the I.R. spectrum of menadione sodium bisulfite.

Fig. 4 the I.R. spectrum of thiamine hydrochloride.

20. Fig. 5 the I.R. spectrum of the mole to mole menadione bisulfite-thiamine adduct.

From the comparison of the above spectra it evidently appears that the BMW spectrum has a strong absorption band at 1740 cm^{-1} . This band does not appear in any other spectrum particularly neither in

the adduct 2-1 spectrum nor in the physical mixture spectrum of the two active components.

The spectrum of physical mixture (figure 2) corresponds, by good approximation, to the summation of the spectra of the single
5. components.

The elementary analysis gives the following results:

- C = 52,87% (the theoretical value for the adduct BMW = 52,84%)
H = 4,72% (the theoretical value for the adduct BMW = 4,70%)
N = 7,25% (the theoretical value for the adduct BMW = 7,25%)
10. O = 22,75% (the theoretical value for the adduct BMW = 22,77%)
S = 12,41% (the theoretical value for the adduct BMW = 12,44%)

Therefore the obtained product perfectly corresponds (the 100%) to the formula $C_{34}H_{36}N_4O_{11}S_3$, which is the desired product. This product is water - as well as chloroform and ethyl alcohol - fairly
15. soluble at a room temperature, namely:

in 100g of water, dissolve 6g of this product
in 100g of chloroform dissolve 0,2g of this product
in 100g of ethyl alcohol dissolve 0,2g of this product
The pH of saturated aqueous solutions is 3,3 at 25°C.

20. Example 2 - Experimental preparation on semi-industrial scale

To a solution of 100g of commercial menadione sodium bisulfite (menadione title 49,5%) and 200g water, 50g of commercial thiamine hydrochloride (title 97%) are added, within five minutes under stirring, at a temperature not higher than 30°C. After 5 minutes,
25. since the thiamine addition has been completed, the product starts

precipitating. The stirring is protracted during 30 minutes and then the slurry is cooled to 0°C, filtered without washing and, after vacuum drying to 40°C, 100g of white solid are obtained, this latter having the following analysis:

5. menadione = 43,66%
 thiamine (as hydrochloride)..... = 42,77%
 NaCl = 2,0%
 H₂O = 0
 or: the adduct BMV = 98,0%
10. NaCl = $\frac{2,0\%}{100,0\%}$

The yield is 88,2%

Example 3 - Pilot preparation

This example represents a model for an industrial preparation of an
 15. adduct (BMV) according to the present invention.

A solution of 2 kg of commercial menadione sodium bisulfite (menadione title 51,29%) in 4 kg of a liquid, consisting of 2 kg of water and 2 kg of a filtrate recycled from a preceding preparation, is prepared.

20. To this solution, 1 kg of commercial thiamine hydrochloride (97%), within 10 minutes, at a temperature not higher than 30°C, is added; the thiamine hydrochloride dissolves and, after about 10 minutes, the product BMV starts precipitating. After 30 minutes of curing, the product is cooled to 0°C and centrifugated without washing.
25. After vacuum drying at 40°C, 2,25 kg of product having the following analysis are obtained:

BMW = 96,6% (menadione 43%;thiamine hydrochloride equivalent=42%)

NaCl = 3,4%

H₂O = 0%

A product is so obtained which is pure up to 96,6%.

5. Example 4 - Stabilization tests of the product of this invention in feeds

In order to determine the stability of the product according to this invention, several mixtures of the adduct to be tested with aluminum silicate, having 10,4% moisture, (as a support) were
10. prepared.

The tested mixtures were the following:

- A) = 1,5g product BMW + 50g aluminum silicate
- B) = 1,5g menadione sodium bisulfite + 50g aluminum silicate
- C) = 0,75g thiamine hydrochloride + 50g aluminum silicate

15. The mixtures were placed in sealed containers and maintained at 55°C in a thermostat for 3 different periods of times. It was observed:

- after 1 day (24 hours): in the sample were still present:

(A) 95,3% of the initially present menadione

20. 63,7% of the initially present thiamine hydrochloride

(B) 70,2% of the initially present menadione

(C) 47,2% of the initially present thiamine hydrochloride

- after 3 days (72 hours): in the sample where still present:

(A) 92,0% of the initially present menadione

25. 28,7% of the initially present thiamine hydrochloride

(B) 23,1% of the initially present menadione

(C) 16,5% of the initially present thiamine hydrochloride

Example 5 - Comparison tests of the BMW product stability, present in integrator mixtures for zootechny as compared with menadione sodium bisulfite MSB and a thiamine hydrochloride in the same conditions:

5. The tests were carried out on three integrator types having the same percentage composition, which corresponds to a standard industrial use, namely:

oligoelement mixture	10,0%
vitamin mixture (vitamins different from K and B vitamin)	3,5%
10. coline HCl 50%	20,0%
support q.sb.	to 100

The support differs as following

A) Grape peels (with moisture)	6,80%
B) Maize gluten flour (with moisture)	8,40%
15. C) Grape peels + CaCO_3 (2:1) (with moisture)	5,40%

Practically an integrator generally contains the herein claimed adduct BMV in a ratio of 2,5g to kg integrator.

Sample	% of the initially present monodione still present in the sample after the reported days										% of initially present thiamine hydrochloride still present in the sample after the reported days									
	← At ambient temp. →										← At ambient temp. →									
	30	60	90	150	30	60	90	150			30	60	90	150	30	60	90	150		
A + MSB	66	44	41	36	24	13	11	8			/	/	/	/	/	/	/	/		
B " "	69	44	40	26	24	13	11	9			/	/	/	/	/	/	/	/		
C " "	67	55	53	47	54	38	34	16			/	/	/	/	/	/	/	/		
A + adduct BMV	75	74	68	63	74	72	59	49			90	91	83	67	94	90	86	59		
B + " "	79	73	69	58	68	57	65	37			85	82	77	62	74	67	62	60		
C + " "	74	71	86	75	75	67	68	53			88	84	80	73	88	84	78	59		
A + B ₁ HCl	/	/	/	/	/	/	/	/			62	57	42	30	60	47	24	22		
B + " "	/	/	/	/	/	/	/	/			71	55	44	42	55	48	46	35		
C + " "	/	/	/	/	/	/	/	/			50	44	40	40	45	35	32	30		

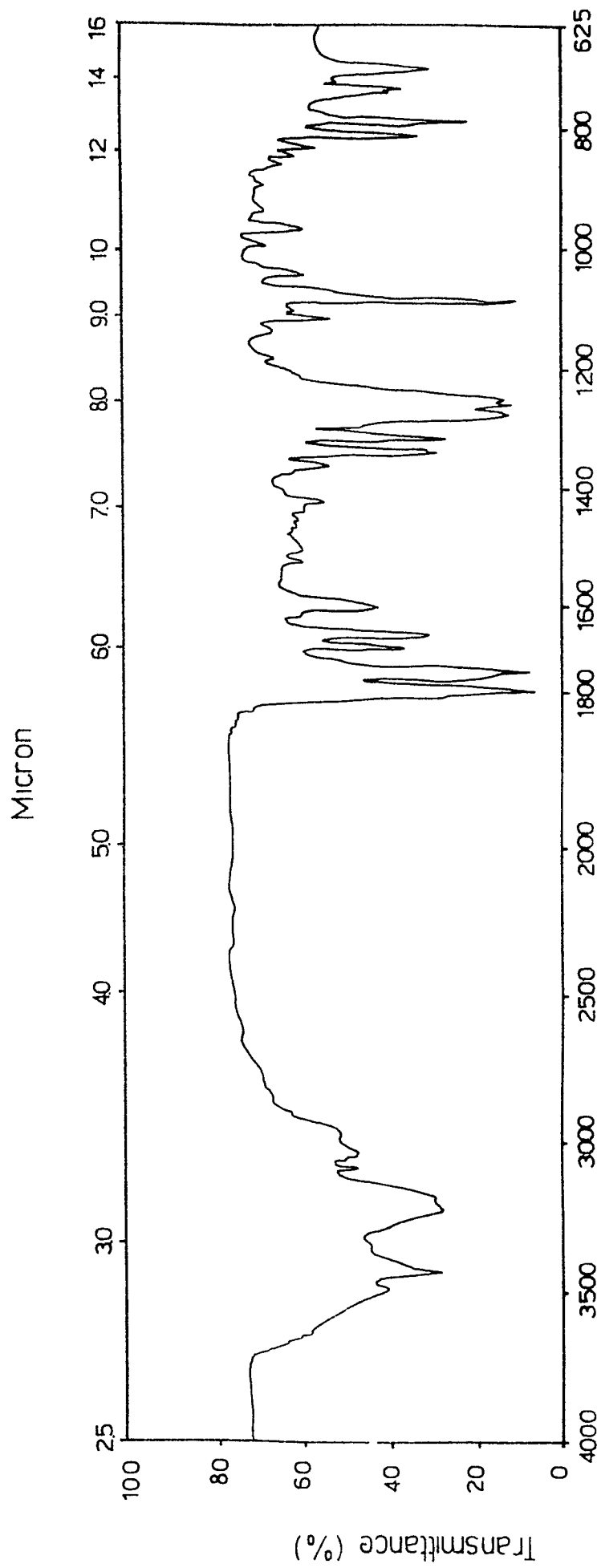
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C L A I M S

1. An adduct of menadione with thiamine characterized by the fact that it comprises two parts of menadione and one part of thiamine hydrochloride, and by the fact that both parts are mutually stabilizing.
5. 2. Adduct according to claim 1, having the general formula $C_{34}H_{36}N_4O_{11}S_3$, and containing 44,56% b.w. of menadione ($C_{11}H_8O_2$) and 43,64% b.w. of thiamine chlorhydrate ($C_{12}H_{18}Cl_2N_4O_5S$).
3. Feed integrator which contains an adduct according to claim 1 in ratio of 2,5g a kg of integrator.

FIG.1



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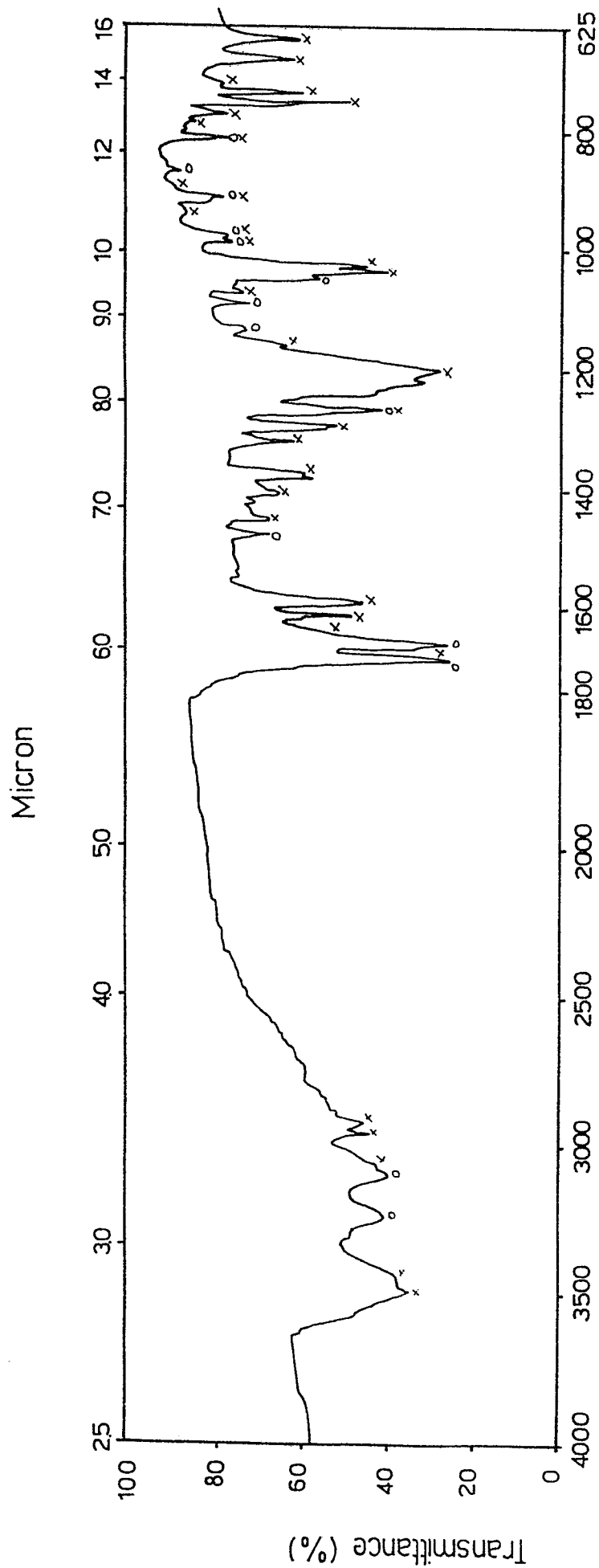
sample: BMV

Date 5-21-82

Concentration

1% in KBr

FIG.2



sample:

Menadione sodium bisulfite
and Thiamine hydrochloride

Concentration

1% in KBr

Wave N.

Date: 5-21-82

Remarks:

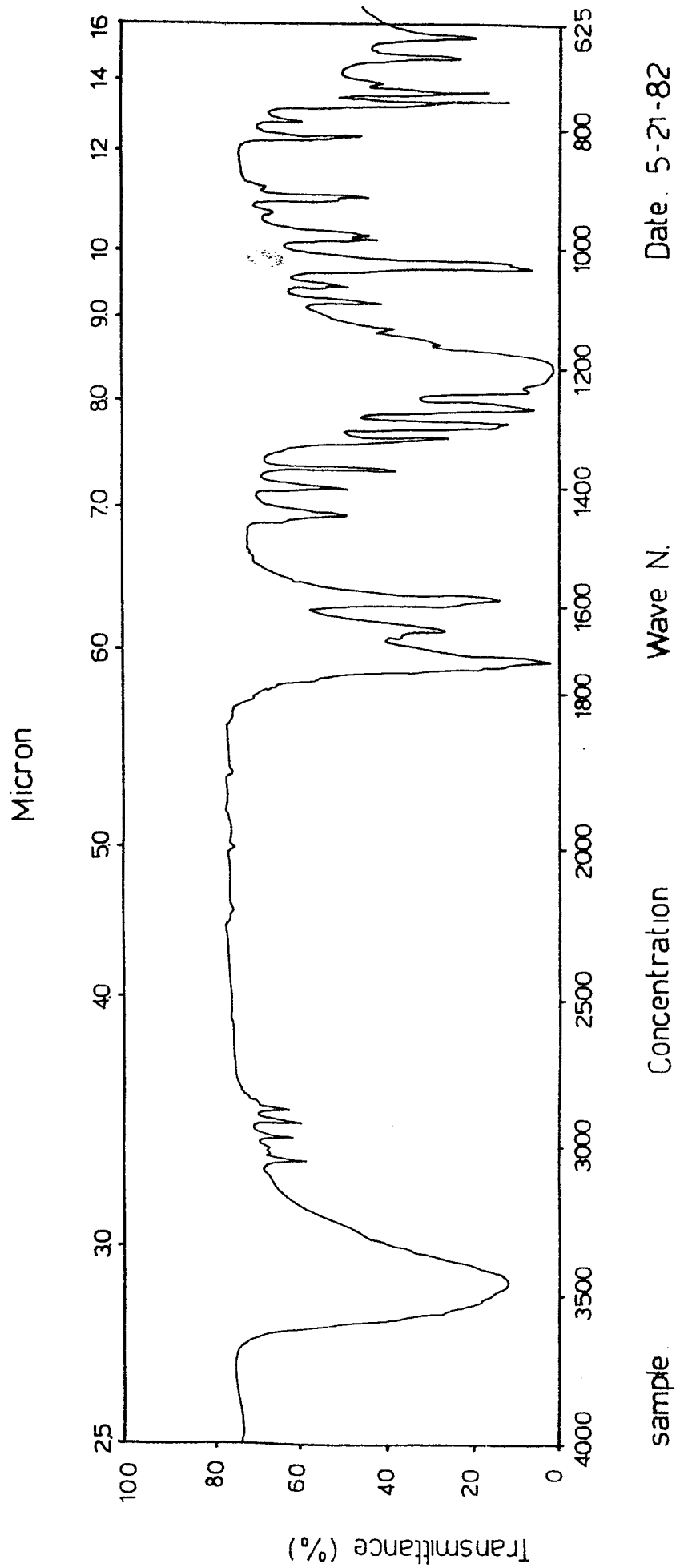
o = B₁

x = K₃

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FIG. 3



Date: 5-21-82

Wave N.

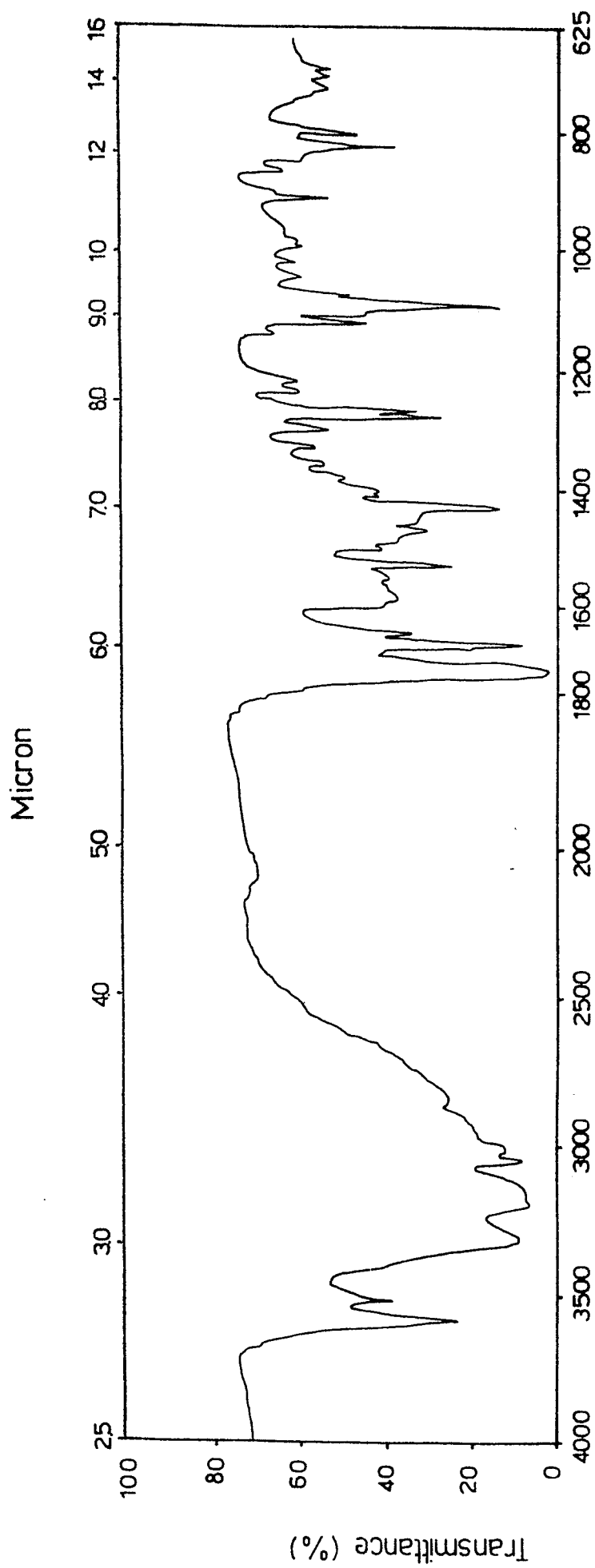
Concentration

1% in KBr

sample

Menadione sodium
bisulfite

FIG.4



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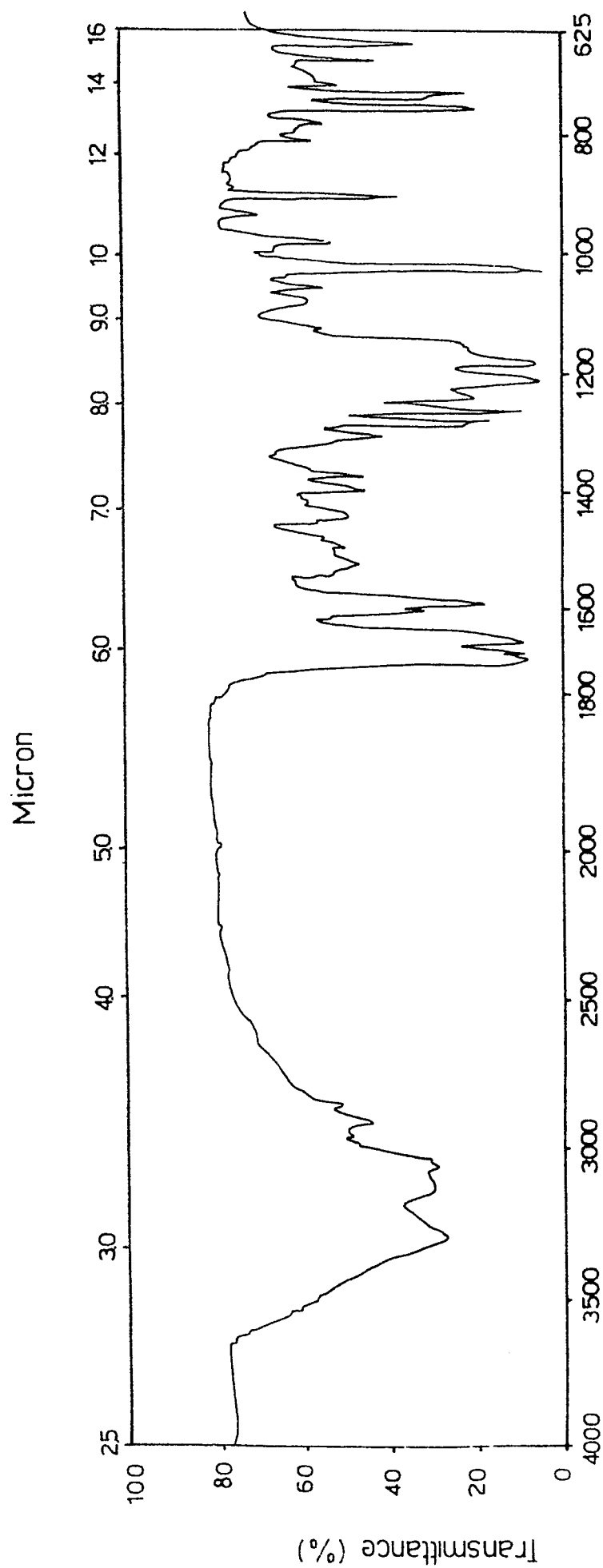
sample: Thiamine hydrochloride

Concentration 1% in KBr

Date: 5-21-82

Wave N.

FIG. 5



Date 5-21-82

Wave N.

Concentration

1% in KBr

sample
Menadione Thiamine
Bisulfite
(Molar ratio 1:1)

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 3)
A	GB-A- 685 667 (BAYER) * Page 1 * -----	1,3	C 07 D 415/00 A 23 K 1/16
			TECHNICAL FIELDS SEARCHED (Int. Cl. 3)
			C 07 D 415/00
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 03-10-1983	Examiner FRANCOIS J.C.L.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			